Inflammation and fatness in adolescents with and without Down Syndrome. UP&DOWN Study

Journal:	*Journal of Intellectual Disability Research*
Manuscript ID	Draft
Manuscript Type:	Original Manuscripts
Keywords:	inflammatory biomarkers, fatness, adolescents, Down syndrome

Abstract

Background: The main objective of this study was to describe the inflammatory status of adolescents with Down Syndrome (DS) and their relationship with fatness. **Methods:** 95DS adolescents (44.2% girls) and a control group of 113 adolescents (47.8% girls), 11-18 years, from the UP&DOWN Study participated. Serum C-reactive protein (CRP), C3 and C4 complement factors, total proteins, interleukin-6 (IL-6), tumor necrosis factor-α (TFN-α), insulin, cortisol, leptin, adiponectin, galactin-3 and visfatin were analyzed; HOMA-index was calculated. Body fat indicators: weight, height, waist circumference and skinfold thicknesses were measured. Birth weight was obtained by questionnaire. BMI, waist-to-height-ratio (WHtR) and body fat percentage (BF%) were calculated. **Results:** DS group showed higher levels of BMI, WHtR, waist circumference, BF%, and lower birth weight than controls ($p<0.001$). In the general lineal model in the total sample, WHtR was positively associated with C3 and C4 ($p<0.001$) as well as with leptin levels ($p=0.015$). BF% was positively associated with total proteins ($p=0.093$) and leptin levels ($p<0.001$). DS was positively associated with total proteins ($p<0.001$), C3 ($p=0.047$) and C4 ($p=0.019$). Despite the higher levels of fatness found in DS group, no direct association was found between BF% and leptin levels, opposite to the control group. **Conclusions:** these findings suggest that abdominal obesity should be controlled in adolescents due to its relationship with acute phase-inflammatory biomarkers, but especially on DS adolescents who may show a peculiar metabolic status according to their relationship between fatness and inflammatory biomarkers.

Key words: inflammatory biomarkers; fatness; adolescents; Down Syndrome.

Background

Down Syndrome (DS) is a human genetic disorder due to the triplication of chromosome 21 that is associated with several chronic pathologies, such as cardiovascular diseases, obesity, diabetes mellitus or Alzheimer (Roizen & Patterson 2003), together with a variety of dysmorphic physical characteristics, and immunodeficiency (Kusters et al. 2009). Autoimmune diseases occur more frequently in individuals with DS than in those without DS. However, the etiology of this immunological disorder has not been fully described (Santos-Silva CR, Biselli-Périco JM et al. 2016).

Inflammation is a natural defense mechanism of body tissues from the immune system. Nevertheless, chronic inflammation is associated with the development of cardiovascular and metabolic diseases, such as atherosclerosis and type 2 diabetes. Fat tissue excess is related to a low-grade inflammation status that produces immune-related mediators, like adipokines that play an important role in sugar and fat metabolism, immunity and cardiovascular function (Smekal & Vaclavik 2017).

Inflammatory conditions seem to have a crucial role in DS. However, this status of low-grade inflammation in DS has to be confirmed in humans (Fructuoso et al. 2018). Therefore, the main aim was to describe the inflammatory status of adolescents with Down Syndrome (DS) and their relationship with fatness.

Methods

Sample and study design

The UP & DOWN Study is a two-year follow-up study designed to assess the impact over time of physical activity and sedentary behaviors on health indicators, such as physical fitness, metabolic and cardiovascular disease risk factors, inflammatory biomarkers and mental health,

as well as to identify the psycho-environmental and genetic determinants of physical activity in a Spanish sample of adolescents with and without DS (Castro-Piñero et al. 2014). Parents and school supervisors were informed by letter about the nature and purpose of the study, and written informed consent was obtained.

For the current study, we included initial data collected from September 2011 to June 2012 in Madrid (Spain), all the participants aged between 11 and 18 years with available blood samples were included. The sample included a total of 95 DS adolescents (44.2% girls) (DS group) and 113 adolescents (47.8% girls) (control group). The study protocols were approved by the Ethics Committee of Puerta de Hierro Hospital (Madrid, Spain) and the Bioethics Committee of the Spanish National Research Council (Madrid, Spain). The study conforms to recognized Declaration of Helsinki standards.

Inflammatory biomarkers

Fasting blood samples were collected early in the morning. In all cases, blood was extracted from the cubital vein according to the established protocol (Castro-Piñero et al. 2014). Twelve key biomarkers involved in the inflammatory process were analyzed for this study: C-reactive protein (CRP, mg/L), C3 (C3, mg/dL) and C4 component factors (C4, mg/dL) by turbidimetry (AU2700 Olympus Analyzer; Olympus UK Ltd, Watford, UK); total proteins (g/L) by colorimetric assay (AU2700 Olympus analyser); tumor necrosis factor-α (TNF-α pg/mL), interleukin-6 (IL-6, pg/mL), adiponectin (x10^6 pg/mL), insulin (pg/mL) and leptin (pg/mL) by Immunoassay (xMAP Techonology) using a kit (5 + 1) plex: 171B5006M Bio-Plex Human IL-6 set; 171B5026M Bio-Plex Human TNF-α set; 171D50001 Bio-Plex Human Cytokine Stds; 171-A7003M Bio-plex Pro Human Diabetes Adiponectin Assay; YB0000002Y Bio-Plex Human Diabetes 3-Plex Assay; visfatin (ng/mL) by Enzyme-linked ImmunoSorbent Assay (Human visfatin Elisa kit; Cusabio Biotech); galactin-3 (pg/mL) by enzyme-Linked

ImmunoSorbent Assay (Omnikine TM Human Galectin-3 Elisa Kit, Assay biotech) and cortisol (pg/mL) by enzyme-Linked ImmunoSorbent Assay (ARBOR ASSAYS kit). Also, homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by the following equation: Fasting glucose (mmol/L)×fasting insulin (mIU/L)/22.5.

Fatness measurements

Body fat indicators were assessed following standardized procedures (Ruiz et al. 2011). Weight was measured using an electronic scale (model SECA 701, Hamburg, Germany) and height by using a telescopic height-measuring instrument (model SECA 220, Hamburg, Germany). Body mass index was expressed as kg/m^2. As indices of abdominal obesity, both waist circumference [indicator of abdominal body fat, was measured with a non-elastic tape (SECA 200; SECA, Hamburg, Germany) at the level of the narrowest part of the torso] and waist-to-height ratio (WHtR) (indicator of abdominal body fat, was calculated as waist circumference/height) were used for this purpose. Body fat percentage (BF%) was calculated from triceps and subscapular skinfold thicknesses, that were measured with a Holtain caliper, using the Slaughter's equations (Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD 1988). These equations accurately predict body fat by these skinfolds in both populations (González-Agüero et al. 2011; Rodríguez et al. 2005). Birth weight was obtained by a questionnaire. The whole protocol of the study had been previously described (Castro-Piñero et al. 2014).

Statistical analysis

Differences between groups (DS *vs* control) in age, birth weight, BMI, waist circumference, WHtR, BF%, C3 and C4 complements, CRP, total proteins, IL-6, TNF-α, insulin, HOMA-IR, cortisol, leptin, adiponectin, galactin-3 and cortisol were calculated using Student's t-test for independent samples (continuous variables). Kolmogorov-Smirnov test was performed to

confirm the normal distribution of the variables. To normalize galactin-3 variable, natural logarithm was calculated. IL-6 and TNF-α did not present a normal distribution either with the calculation of box transformations.

Chi square test was analyzed to determine the differences between groups of visfatin detectable levels. In order to minimize the effect of multicollinearity for each biomarker, the most explicative covariates (age, gender, control or DS group, WHtR, BMI group, BF% and birth weight) were previously identified by a Stepwise Regression Analysis. Afterwards, a General Linear Model (GLM) was implemented to each biomarker from these fixed factors: DS, gender and BMI groups; and the following covariates: BF%, WHtR, birth weight and age.

The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 24.0 for WINDOWS; SPSS INC, Chicago) and the level of significance was set at $p<0.05$.

Results

Characteristics of the total sample are shown in Table 1. DS group showed higher levels of BMI, waist circumference, WHtR, and BF% while lower birth weight in comparison with the control group ($p<0.001$). Regarding inflammatory biomarkers, DS group showed higher levels of CRP, C3 and C4 complement factors, total proteins and visfatin while lower levels of adiponectin, insulin and galactin-3 than the control group ($p<0.05$).

Visfatin levels were below the level of detection in 64.5% of the total sample, these levels were only detected in 44 (38.9%) adolescents from the control group and 23 (24.2%) DS adolescents ($p=0.017$).

In the GLM, relationships between some body fat indicators and inflammatory biomarkers were found. WHtR was positively associated with C3 and C4 complement factors and leptin levels. The BF% was positively associated with leptin levels (Table 2).

The feature to show this genetic disorder due to the triplication of chromosome 21 (DS group) seems to be related to higher levels of C3 and C4 complement factors and total proteins (Table 2). In DS subjects no direct association was found between BF% and leptin levels, opposite to the results found in the control group (Figures 1 and 2).

Discussion

This study provides original and useful information about the relationship between body fat indicators and metabolic and inflammatory status in healthy adolescents with DS through the evaluation of fatness and inflammatory biomarkers in this population.

According to our knowledge, the scientific literature is scarce regarding the immune system in the population with DS. Some studies have assessed some immune biomarkers in a relatively low number of subjects (<43) with DS (Santos-Silva CR, Biselli-Périco JM et al. 2016; Corsi et al. 2009; SN et al. 2008) or in other studies showing conjunctly other diseases (Rohrer TR, Hennes P & Dost A, Grabert M, Rami B, Wiegand S 2010; Magni et al. 2004). On the other hand, the number of biomarkers analyzed in this study is much wider than in other studies. Nowadays, DS has been demonstrated to reveal an anthropometric dimorphism associated with their trisomy 21 (Real de Asua et al. 2014), what is clearly found in our results since practically all the body fat indicators determined, such as BMI, waist circumference, WHtR and BF%, showed higher values than the control group. These findings coincide with the results reported by other authors in both children and adolescents (Basil et al. 2016; Bertapelli et al. 2016), as well as in adults (Leti et al. 2015; Nespoli L, Burgio GR, Ugazio AG 1993). This outcome is in

agreement with the characteristics of DS subjects who suffer from overweight and obesity (Bertapelli et al. 2016), and also from abdominal obesity (Real de Asua et al. 2014).

Despite the evaluation of the immune system in DS population acquired certain interest few years ago (Nespoli L, Burgio GR, Ugazio AG 1993), in our opinion these studies lacked enough depth, especially when DS subjects are healthy and young. Therefore, our study aimed to evaluate the inflammatory status of this population with a battery of inflammatory biomarkers of acute phase, such as CRP, C3, C4 and total proteins. All these markers showed higher levels in the DS group than in the control group. Other studies have found similar results related to C3 and C4 complement factors, but in adult population with DS and with other pathologies (Nanjo et al. 2014). However, to our knowledge there are not studies on healthy youth with DS. All these molecules analyzed in our study are considered early cardiovascular risk markers, whose high values are indicative of low-grade inflammation, especially in adolescents with overweight and obesity. We have found a positive relationship between abdominal obesity (assessed by WHtR) and C3 and C4 complement factors (Table 2) in the total adolescents, both with and without DS, that means both biomarkers are related to body fat distribution, in agreement with previous results evaluating the excess of abdominal fat in children and adolescents without DS (Warnberg et al. 2018). On the other hand, the DS group showed higher levels of total proteins than controls (Tables 1,2). Total protein values include serum albumin and globulins that were also assessed since DS subjects are more prone to suffer from infectious diseases (Santos-Silva CR, Biselli-Périco JM et al. 2016). According to Mahan et al. (Mahan LK, Escott-Stump, S 2013) this result could be related to an increase of globulins revealing an immune response triggered by an infectious agent. On the other hand, an association between total proteins and lean body mass has also been observed in adults with renal treatment (Gallar-Ruiz et al. 2012). Nevertheless, we must highlight that no infections were detected in the DS

group assessed in the current study, therefore, the evaluation of all markers, as a whole, is necessary to understand the real inflammation status of this population.

Due to the higher levels of body fat showed by adolescents with DS, we included the assessment of some molecules such as insulin and HOMA-IR, cortisol, leptin, adiponectin, galactin-3 and visfatin in order to evaluate their impact at the metabolic level. In addition, it is important to highlight that DS subjects show an increased risk of both type 1 (Rohrer TR, Hennes P & Dost A, Grabert M, Rami B, Wiegand S 2010) and type 2 diabetes (Helguera et al. 2013).

Adolescents with DS showed lower insulin concentration and a trend towards lower HOMA-IR values in comparison with controls (Table 1), according to other studies performed in children and adolescents (Bertapelli et al. 2016) or adults (Leti et al. 2015) with DS and also in validated DS mice models (Peiris et al. 2016). According to Rohrer et al. (Rohrer TR, Hennes P & Dost A, Grabert M, Rami B, Wiegand S 2010), youth with DS use less insulin since they seem to have a better metabolism control. On the other hand, HOMA-IR remained unmodified between both groups (Table 1), in agreement with other authors (SN et al. 2008). However, a relationship between obesity and hyperinsulinemia has been found in children with DS (Basil et al. 2016), although there is not a consistent association.

Cortisol is well-known to be a steroid hormone that is produced in the hypothalamic-pituitary-adrenal axis and has been positively associated with adipose tissue and low-grade inflammation, especially in obese subjects (Smekal & Vaclavik 2017). However, in our sample cortisol levels remained similar in both groups (DS and control) even though DS adolescents showed higher BMI and BF% than the control group. This result is not in accordance with that found in another study (Bricout et al. 2008) reporting lower levels of cortisol in young adults (22.5 years old) with DS in comparison with a control group. In addition, since cortisol levels raise with age in

the adult general population (Pal & Singh 2014), we could elucubrate that this result could be due to the different age range in both studies.

Leptin is another adipokine assessed in the current study as a hormone involved in food and appetite regulations. Leptin levels remained unmodified between both groups in the current study (Table 1), opposite result to that found in youth (Corsi et al. 2009; SN et al. 2008) and adults (Leti et al. 2015) with DS, despite leptin has been associated with high BMI in prepuberal youth with DS (Magni et al. 2004). Indeed, this result coincides with our findings in the general lineal model, where trisomy 21 did not present the same direct relationship between body composition and WHtR and leptin levels in comparison with the control group (Table 2, Figures 1 y 2). May be, this is the reason why other studies have accepted a lack in the knowledge of the relationship between adiposity and inflammation in DS subjects (Fructuoso et al. 2018).

Adiponectin is a hormone involved in glucose regulation and fatty acid catabolism, with cardioprotective characteristics and probably with an anti-inflammatory effect (Shetty et al. 2009). In the current study, DS adolescents showed lower adiponectin levels than the control group (Table 1). These results are in agreement with Martínez-Gómez et al. (Martinez-Gomez et al. 2012) and Turer et al. (Turer et al. 2011) studies in healthy adolescents and adults, respectively, and in a smaller sample of children with DS (Tenneti et al. 2017). However, such inflammatory risk seems to be diminished with aging, decreasing also the cardiovascular risk (Corsi et al. 2009). Maybe this fact could contribute to a lower prevalence of cardiovascular mortality in the DS population (Uppal et al. 2015).

The high percentage of congenital coronary alterations found in the DS population (Diogenes et al. 2017) acquires a great interest, despite not being the main cause of mortality (Uppal et al. 2015). Therefore, we included the assessment of galactin-3 as a marker of cardiovascular pathologies (de Boer et al. 2012; De Boer et al. 2014; Vassalle et al. 2017). In the current study,

adolescents with DS showed lower levels of galactin-3 than the control group (Table 1). However, opposite results in Ts65Dn have been found in adult mice, a validated trisomy 21 model (Fructuoso et al. 2018). These controversial results could be due to the different age-range in these studies since galactin-3 increases with age in general population (de Boer et al. 2012).

Visfatin is secreted by visceral fat (Smekal & Vaclavik 2017), being usually increased in cardiovascular diseases (Turer et al. 2012), and related to insulin resistance and type 2 diabetes (Chen et al. 2006) in general population. DS subjects showed higher visfatin values than the control group (Table 1). In the same way, several authors have pointed out a positive association between visfatin and BMI as well as WHtR index in DS adolescents (Blüher et al. 2017); and also with BF% in children (García-Hermoso et al. 2017) and adults (Turer et al. 2012) without DS. It is highlighted that despite most DS subjects had undetectable levels of visfatin, when these levels were detectable, they were higher in DS subjects than in the control group (Table 1).

Finally, our findings demonstrate that adolescents with DS show higher body fat indicators, which lead to a higher risk of obesity. Furthermore, abdominal obesity, through WHtR assessment, is positively related to inflammatory and cardiovascular risk biomarkers such as C3 and C4 complement factors and leptin. Therefore, in agreement with other authors, this anthropometric index should be taken into account in children and adolescents, being especially important in DS population where exist other idiosyncratic factors such as trisomy 21 that may get worse an inflammatory status. However, in view of these results we could interpret that this chromosomic alteration may be a metabolic modulator effect on variables such as leptin. Thus, it seems that adolescents with DS may show a peculiar inflammatory and metabolic behavior, different from control subjects at the same age.

The principal limitation of the current study is the cross-sectional design, which does not allow us to read any conclusion about the causal direction of associations. Likewise, the sample could not be adjusted by pubertal development due to complications found in the self-reported data collection in the DS group. However, as strength, the relative large sample of adolescents with DS and the large number of biomarkers analyzed should be highlighted, in the context of few previous studies assessing the interactions between inflammatory biomarkers and body fat indicators in adolescents with DS.

Indeed, the knowledge of the relationship between body fat indicators and inflammatory biomarkers in DS could be a big help in the control of associated pathologies to the DS chromosomic alteration. In future investigations, the assessment of molecules such as C3 and C4 complement factors, total proteins, visfatin and leptin could be helpful to understand their different behavior in youth with DS. In addition, interventions in subjects with DS addressed to enhance their body composition must be performed in order to avoid any inflammation status risk, especially nowadays when their life expectancy is increasing.

References

Basil, J.S. et al., 2016. Retrospective study of obesity in children with down syndrome. *Journal of Pediatrics*, 173, pp.143–148. Available at: http://dx.doi.org/10.1016/j.jpeds.2016.02.046.

Bertapelli, F. et al., 2016. Overweight and obesity in children and adolescents with Down syndrome—prevalence, determinants, consequences, and interventions: A literature review. *Research in Developmental Disabilities*, 57, pp.181–192. Available at: http://dx.doi.org/10.1016/j.ridd.2016.06.018.

Blüher, S. et al., 2017. Cardiometabolic risk markers, adipocyte fatty acid binding protein (aFABP) and the impact of high-intensity interval training (HIIT) in obese adolescents. *Metabolism: Clinical and Experimental*, 68, pp.77–87. Available at: http://dx.doi.org/10.1016/j.metabol.2016.11.015.

de Boer, R.A. et al., 2012. The fibrosis marker galectin-3 and outcome in the general population. *Journal of Internal Medicine*, 272(1), pp.55–64.

De Boer, R.A. et al., 2014. Galectin-3: A modifiable risk factor in heart failure. *Cardiovascular Drugs and Therapy*, 28(3), pp.237–246.

Bricout, V.A. et al., 2008. Are hormonal responses to exercise in young men with Down's syndrome related to reduced endurance performance? *Journal of Neuroendocrinology*, 20(5), pp.558–565.

Castro-Piñero, J. et al., 2014. Follow-up in healthy schoolchildren and in adolescents with DOWN syndrome: Psycho-environmental and genetic determinants of physical activity and its impact on fitness, cardiovascular diseases, inflammatory biomarkers and mental health; The UP&DOWN Study. *BMC Public Health*, 14(1), pp.1–12.

Chen, M.-P. et al., 2006. Elevated Plasma Level of Visfatin/Pre-B Cell Colony-Enhancing Factor in Patients with Type 2 Diabetes Mellitus. *The Journal of Clinical Endocrinology & Metabolism*, 91(1), pp.295–299. Available at: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2005-1475.

Corsi, M.M. et al., 2009. Adipocytokines in Down's syndrome, an atheroma-free model: Role of adiponectin. *Archives of Gerontology and Geriatrics*, 48(1), pp.106–109.

Diogenes, T.C.P. et al., 2017. Gender differences in the prevalence of congenital heart disease in Down's syndrome: A brief meta-analysis. *BMC Medical Genetics*, 18(1), pp.1–5.

Fructuoso, M. et al., 2018. Increased levels of inflammatory plasma markers and obesity risk in a mouse model of Down syndrome. *Free Radical Biology and Medicine*, 114(June 2017), pp.122–130.

Gallar-Ruiz, P. et al., 2012. Composición corporal en pacientes en hemodiálisis: Relación con la modalidad de hemodiálisis, parámetros inflamatorios y nutricionales. *Nefrologia*, 32(4), pp.467–476.

García-Hermoso, A. et al., 2017. Exercise, adipokines and pediatric obesity: a meta-analysis of randomized controlled trials. *International Journal of Obesity*, 41(4), pp.475–482. Available at: http://www.nature.com/doifinder/10.1038/ijo.2016.230.

González-Agüero, A. et al., 2011. Accuracy of prediction equations to assess percentage of body fat in children and adolescents with Down syndrome compared to air displacement plethysmography. *Research in Developmental Disabilities*, 32(5), pp.1764–1769.

Helguera, P. et al., 2013. Adaptive downregulation of mitochondrial function in down syndrome. *Cell Metabolism*, 17(1), pp.132–140. Available at: http://dx.doi.org/10.1016/j.cmet.2012.12.005.

Kusters, M.A.A. et al., 2009. Intrinsic defect of the immune system in children with Down syndrome: A review. *Clinical and Experimental Immunology*, 156(2), pp.189–193.

Leti, T. et al., 2015. Difference of catecholamine responses to exercise in men with trisomy 21, with or without chronotropic incompetence. *Physiology and Behavior*, 142, pp.97–103. Available at: http://dx.doi.org/10.1016/j.physbeh.2015.02.007.

Magni, P. et al., 2004. Free and bound leptin in prepubertal children with Down's syndrome and different degrees of adiposity. *Eur J Clin Nutr*, 58(11), pp.1547–1549. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15162134%5Cnhttp://www.nature.com/ejcn/journal/v58/n11/pdf/1602000a.pdf.

Mahan LK, Escott-Stump, S, R.J., 2013. *Krause Dietoterapia*,

Martinez-Gomez, D. et al., 2012. Associations of physical activity and fitness with adipocytokines in adolescents: The AFINOS study. *Nutrition, Metabolism and Cardiovascular Diseases*, 22(3), pp.252–259.

Nanjo, S. et al., 2014. Immune-mediated Protein-losing Enteropathy with Down Syndrome. *Internal Medicine*, 53(20), pp.2301–2305. Available at: http://jlc.jst.go.jp/DN/JST.JSTAGE/internalmedicine/53.1980?lang=en&from=CrossRef&type=abstract.

Nespoli L, Burgio GR, Ugazio AG, M.R., 1993. Immunological features of Down's syndrome: a review. *J Intellect Disabil Res*, 37(6), pp.543–51.

Pal, R. & Singh, S.N., 2014. Age-related changes in cardiovascular system , autonomic functions , and levels of BDNF of healthy active males : role of yogic practice.

Peiris, H. et al., 2016. A Syntenic Cross Species Aneuploidy Genetic Screen Links RCAN1

Expression to β-Cell Mitochondrial Dysfunction in Type 2 Diabetes. *PLoS Genetics*, 12(5), pp.1–24.

Real de Asua, D. et al., 2014. Evaluation of the impact of abdominal obesity on glucose and lipid metabolism disorders in adults with Down syndrome. *Research in Developmental Disabilities*, 35(11), pp.2942–2949.

Rodríguez, G. et al., 2005. Body fat measurement in adolescents: Comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. *European Journal of Clinical Nutrition*, 59(10), pp.1158–1166.

Rohrer TR, Hennes P, T.A. & Dost A, Grabert M, Rami B, Wiegand S, H.R., 2010. Down's syndrome in diabetic patients aged <20 years: An analysis of metabolic status, glycaemic control and autoimmunity in comparison with type 1 diabetes. *Diabetologia*, 53(6), pp.1070–1075.

Roizen, N.J. & Patterson, D., 2003. Down's syndrome. *The Lancet*, 361(9365), pp.1281–1289. Available at: http://www.thelancet.com/article/S014067360312987X/fulltext.

Ruiz, J.R. et al., 2011. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *British Journal of Sports Medicine*, 45(6), p.518 LP-524. Available at: http://bjsm.bmj.com/content/45/6/518.abstract.

Santos-Silva CR, Biselli-Périco JM, L.-Z.B., Araujo-Silva W, Santana de Souza JE, C.-B.M. & Goloni-Bertollo EM, P.E., 2016. Differential Expression of Inflammation-Related Genes in Children with Down Syndrome. *Mediators of Inflammation*, 2016.

Shetty, S., Kusminski, C.M. & Scherer, P.E., 2009. Adiponectin in health and disease: evaluation of adiponectin-targeted drug development strategies. *Trends in*

Pharmacological Sciences, 30(5), pp.234–239.

Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, B. DA, 1988. Skinfold Equations for Estimation of Body Fatness in Children and Youth. *Human Biology*, 60(5), pp.709–23.

Smekal, A. & Vaclavik, J., 2017. Adipokines and cardiovascular disease: A comprehensive review. *Biomedical Papers*, 161(1), pp.31–40.

SN, M. et al., 2008. Leptin levels among prepubertal children with Down syndrome compared with their siblings. *Journal of Pediatrics*, 152(3), pp.321–326. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=rzh&AN=105874666&site=ehost-live&scope=site.

Tenneti, N. et al., 2017. Concentrations of leptin, adiponectin and other metabolic parameters in non-obese children with Down syndrome. *Journal of Pediatric Endocrinology and Metabolism*, 30(8), pp.831–837.

Turer, A.T. et al., 2012. Adipose tissue biology and cardiomyopathy: Translational implications. *Circulation Research*, 111(12), pp.1565–1577.

Turer, A.T. et al., 2011. Adipose tissue mass and location affect circulating adiponectin levels. *Diabetologia*, 54(10), pp.2515–2524.

Uppal, H., Chandran, S. & Potluri, R., 2015. Risk factors for mortality in Down syndrome. *Journal of Intellectual Disability Research*, 59(9), pp.873–881. Available at: http://doi.wiley.com/10.1111/jir.12196.

Vassalle, C. et al., 2017. Traditional and new candidate cardiac biomarkers assessed before, early, and late after half marathon in trained subjects. *European Journal of Applied Physiology*, 118(2), pp.1–7. Available at: http://dx.doi.org/10.1007/s00421-017-3783-x.

Warnberg, J. et al., 2018. Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. *American Journal of Clinical Nutrition*, 84(3), pp.505–512. Available at: http://www.ajcn.org/cgi/content/abstract/84/3/505.

Table 1. Description of main variables in both groups, Down Syndrome and control groups

	Control		Down Syndrome		p-value
	n	Mean (SD)[a]	n	Mean (SD)	
Age (years)	113	14.64 (2.16)	95	14.22 (2.00)	0.172
Birth weight (kg)	108	3.16 (0.64)	67	2.77 (0.81)	**<0.001**
BMI[b] (kg/m^2)	112	18.50 (2.77)	95	23.98 (4.24)	**<0.001**
Waist circumference (cm)	113	62.21 (7.88)	95	74.03 (9.72)	**<0.001**
WHtR[c] (cm/cm)	112	0.41 (0.03)	95	0.49 (0.06)	**<0.001**
BF[d] %	113	18.91 (6.12)	91	29.26 (5.44)	**<0.001**
CRP[e] (mg/L)	57	0.90 (2.68)	87	4.49 (9.31)	**0.001**
C3 (mg/dL)	105	91.90 (24.65)	90	119.75 (22.52)	**<0.001**
C4 (mg/dL)	105	20.11 (9.30)	90	30.06 (9.09)	**<0.001**
Total proteins (g/L)	77	58.74 (15.68)	90	72.21 (5.37)	**<0.001**
IL-6[f] (pg/mL)	112	28.18 (25.07)	87	37.75 (46.31)	0.086
TNF-α[g] (pg/mL)	100	78.04 (76.40)	68	71.75 (112.4)	0.666
Insulin (pg/mL)	81	413.5 (368.4)	56	243.9 (347.6)	**0.008**
HOMA-IR[h] (mmol/L*mIU/L)	78	2.34 (2.24)	53	1.57 (2.28)	0.061
Cortisol (pg/mL)	102	197972 (102877)	87	173669 (86454)	0.083
Leptin (pg/mL)	103	7187.7 (6563.6)	77	9169.9 (10800.7)	0.157
Adiponectin (x10^6 pg/mL)	105	14.78 (9.18)	90	11.49 (5.21)	**0.002**
Galactin-3 (pg/mL)	101	3598.9 (3010.6)	87	2453. 7 (1583.5)	**0.001**
Visfatin (ng/mL)	44	0.93 (0.78)	23	1.40 (0.75)	**0.019**

[a] Standard deviation; [b] Body mass index; [c] Waist to height ratio; [d] Body fat; [e] C-reactive protein; [f] Interleukin-6; [g] Tumor necrosis factor-α; [h] Homeostatic model assessment for insulin resistance (HOMA-IR).

*Statistical significance was determined by Student's t-test.

Table 2. Analysis of inflammatory biomarkers in adolescents with and without Down Syndrome. General Lineal Model

Outcome variable	Independent variables	B	Standard error	p-value
C3 Adjusted R Squared=0.347	Control	-9.480	4.749	**0.047**
	DS[b]	0[a]		
	WHtR[c]	195.094	36.940	**<0.001**
C4 Adjusted R Squared=0.280	Control	-4.056	1.899	**0.019**
	DS[b]	0[a]		
	WHtR[c]	58.070	14.773	**<0.001**
Total proteins Adjusted R Squared=0.264	Control	-10.660	0.5	**<0.001**
	DS[b]	0[a]		
	BF[d] %	0.261	0.155	0.093
Leptin Adjusted R Squared=0.213	Control	7286.864	1872.830	**<0.001**
	DS[b]	0[a]		
	BF[d] %	566.268	109.627	**<0.001**
	WHtR[c]	35660.685	14543.828	**0.015**

[a] Down Syndrome; [b] This parameter is set to zero because it is redundant; [c] Waist to height ratio; [d] Body fat.

Figure legends

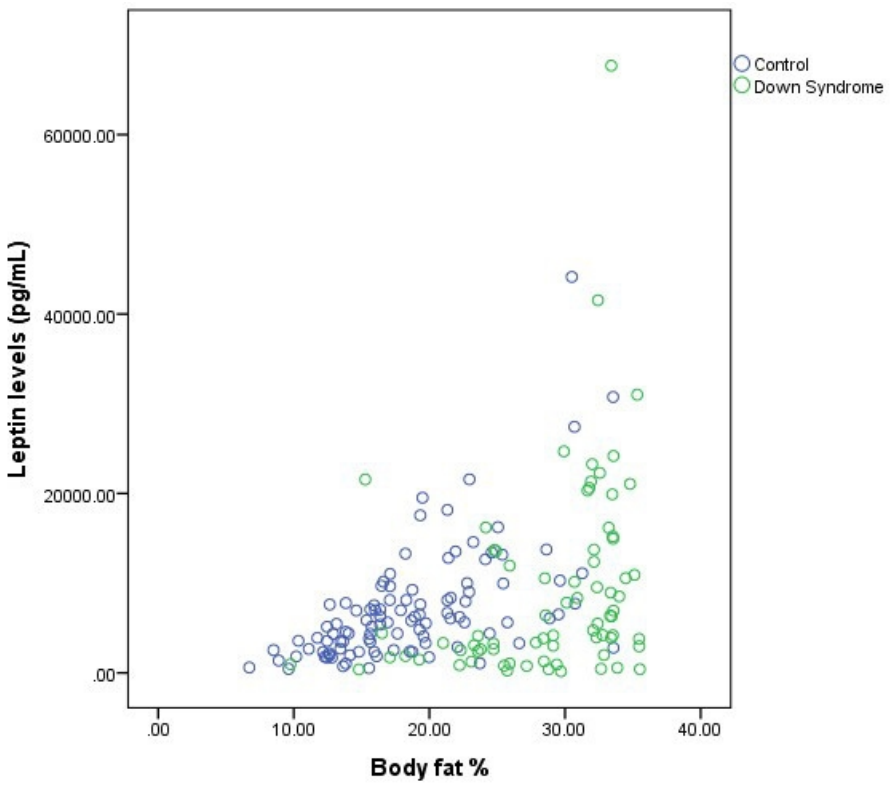
Figure 1

Title: Relationship between leptin levels and body fat percentage in youth with and without Down Syndrome

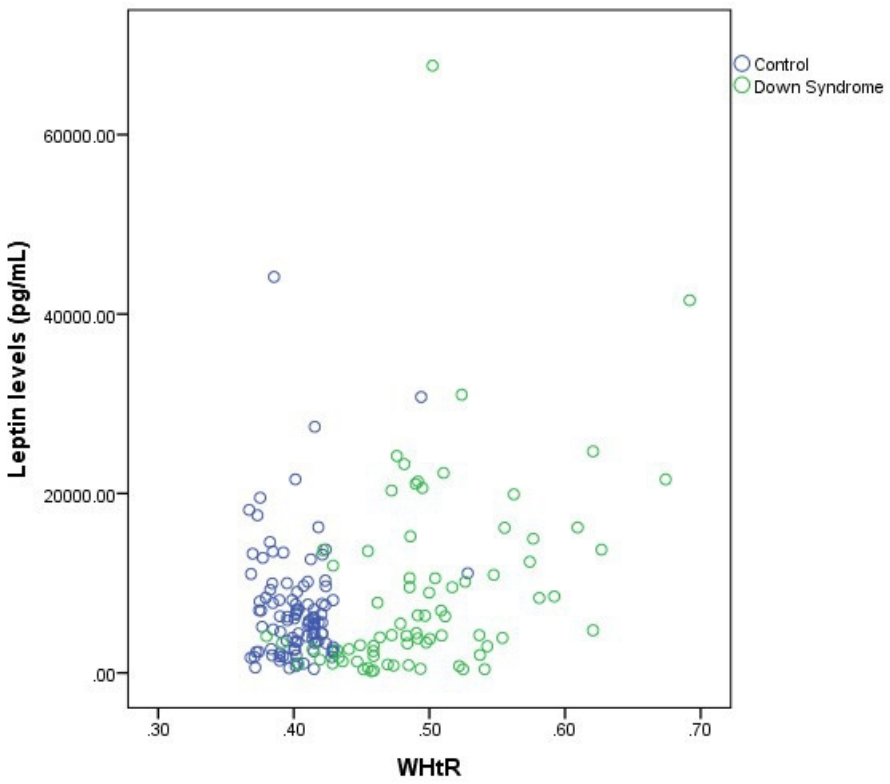
Figure 2

Title: Relationship between leptin levels and WHtR in youth with and without Down Syndrome

Foot of figure: WHtR, Waist to height ratio



165x132mm (96 x 96 DPI)



165x132mm (96 x 96 DPI)